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Nano-particles from *Chaetomium brasiliense* to control *Phytophthora palmivora* caused root rot disease in durian var Montong

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Abstract The antagonistic fungus, *Chaetomium brasiliense* was tested to control *Phytophthora palmivora* causing rot disease of Durian (*Durio zibithenus* L.) var Montong by crude extracts and Nano-particles derived from *Ch. brasiliense*. Crude extracts of antagonistic fungus was tested for antifungal biological activities. The crude extracts from antagonistic fungus with hexane, ethyl acetate and methanol were tested against *P. palmivora.* Crude ethyl acetate from *Ch. brasiliense* gave significantly against *P. palmivora* which the ED_{50} values was 17.46 µg/ml. Testing Nano-particles were tested for antifungal activities. The results showed nano - particles from *Ch. brasiliense* gave effectively significantly inhibition of colony growth and spore production which the ED_{50} values were 1.08 µg/ml, and 8.68 µg/ml respectively. Application of Nano - particles to control the *P. palmivora* causing root rot disease of durian in pot experiment was successfully done. The results showed nano-particles from *Ch. brasiliense* reduced the root rot disease on durain of 40%. The nano–particles from *Ch. brasilense* gave significiantly high plant growth which were 79.5 cm when compared to the non-trated control.

Keywords: Nano-particles, *Chaetomium brasiliense*, *Phytophthora palmivora*, biological control

Introduction

Durian (*Durio zibithenus* L) is originated from the region of Borneo and Sumatra, growing wild in the Malay peninsula, cultivated in a wide region from India to New Guinea four hundred years ago. It was across to Myanmar, and cultivated throug Thailand and South Vietnam (Morton, 2000). The problem for durian cultivation in Thailand faced the root rot which caused by *Phytophthora* spp. It can infect all stages of growing durian trees. The symptoms are appeared as root rot, leaves and stem blight, bark and fruit rot. Chemical fungicides have

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been used to control this disease and trened to lead the negative side effect to the environment. In addition, Phytophthora spp become resistant to those fungicide application eg. metalaxyl and related compounds (Erwin and Ribeiro, 1996). Tye eco-friendly management of *Phytophthora* diseases would be done to reduce the application cost. The application of biocontrol agents against *Phytophthora* rot has become an importance research aspect and is being carried out all over the world (Naqvi, 2004).

Biological control of plant pathogen is known as using antagonists against plant pathogens. The antagonism is the control mechanism to reduce the pathogen onocullum and disease incidence. Antagonism is expressed betweeb two organisms including antibiosis, competition and parasitism (Cook and Baker, 1983). However, thereare many reports indicated that the antagonists can express to against seversal plant pathogens eg such as *Chaetomium* spp; *and Emericella nidulans*. (Xu *et al.,* 2017; Hung, *et al.,* 2015a; Arjona-Girona *et al.,* 2018; Tran *et al.,* 2007; Song *et al.,* 2017).

The objective was to test the nano-particles derived from *Ch. brasiliense* to inhibit *Phytophthora* sp causing root rot in durian var Montong.

Materials and methods

Isolation of pathogen and testing pathogenicity

Phytophthora sp was isolated the durian diseased plant parts eg bark and root rot using tissue transplanting method. The collected diseased samples were washed with sterilized water, and and cut it into small pieces, soaked 1% clorox for 3 min. The piece samples of diseased part was moved to water agar (WA), then incubated at room temperature to observe growing colonies, them gentle moved to PDA to get pure culture. Morphology was identified and observed the characters of fungus under compound microscope. The pathogenicity was tested using detached leaves technique. The agar plugs of *Phytophthora* sp were transferred to wounded leaves. The non -inoculated leaves with steriled agar discs was done to serve as controls, and incubated at room temperature (27-30 ℃) for seven days. The experiment was replicated four times. Disease incidence was calculated as number of infected plants/ total number of tested plants x 100, and disease ratings was evaluated as 0= healthy plants, and 3= seriously infected plants (Soytong, 2010).

Morphological study of Chaetomium brasilense

Chaetomium brasilense used in this reserch study is offered from Assoc. Prof. Dr. Kasem Soytong, Department of Plant Production Technology, Faculty

of Agricultural Technlogy, King Monkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. The culture was grown on patotao dextrose agar and observed morphological characters under compound microscope.

Dual culture test

The experimental design was used as Completely Randomization with four replications. Dual culture was done by followed the methods of Soytong (1992). *Ch. brasilense* and *Phytophthora* sp. were separately cultured on PDA at room temperature (27-30 ℃) for seven days. The 0.5 cm diameter of sterilized cork borer was cut at the peripheral colony in each culture and moved to PDA plates (9 cm). The agar plug of *Phytophthora* sp. was moved to PDA plate on one side of the plate that opposited the agar plug of *Ch. brasilense*. The plug of each fungus was transferred to PDA plate served as the controls. All plates were incubated at room temperature for 30 days. Colony diameter (cm) and conidia production in the dual culture plates and control plates were recorded. The number of conidia was counted by using haemacytometer.

The inhibition of colony growth or conidia production were calculated using the following formula:

$$\% \text{ inhibition} = (A-B) / A \times 100$$

Where, A was the colony diameter or number of conidia produced by the pathogen on the control plates and B was the colony diameter or number of conidia produced by the pathogen in the dual culture plate.

Testing crude extracts of Chaetomium brasilense to against Phytophthora spp in vitro

Crude extracts of *Ch. brasilense* were evaluated to inhibit *Phytophthora* sp. The experimental design was conducted using factorial experiment in Completely Randomization, and repeated four times. Factor A was crude hexane, crude ethyl acetate and crude methanol. Factor B was 0, 10, 50, 100, 500, and 1,000 µg/ml. Each crude extract was poured to 2% dimethyl sulphite and mixed to 30 ml potato dextrose agar, then autoclaved at 121 ℃,15 lbs/inch^2 for 35 minutes. *Phytophthora* sp colony was cut at peripheral colony with sterilized cock borer (0.5 mm). Agar plug of pathogen was transferred to the middle of PDA media in plate incorporated with each and incubated at room temperature (27-30 ℃) until the pathogen growing full plate. Data were recorded as colony diameter, number of conidia. The pathogen colony growth and conidia production were calculated the inhibition using the above formula. Data were statistically computed analysis of variance. Means were compared using Duncan Multiple's Range Test. The effective dose was computed using probit analysis.

In vitro testing nano-particles from Chaetomium brasilense to control root rot disease in durian var Montong

Chaetomium brasilense was cultured in potato dextrose broth (PDB) at room temperature (27-30 C) for 30 days. The biomass was filtered through cheesecloth and air-dried. Fresh and dried biomass were weighted. The biomass was ground in electrical blender, then extracted by adding the same volume of hexane, and kept in stationary phase for 7 days at room temperature. The filtrate was get it through Whatman filter paper, evaporated in rotary vacuum evaporator to yield crude hexane. The remaining marc was consecutively extracted with ethyl acetate and methanol to get crude extracts using the same procedure as hexane to yield crude ethyl acetate (EtOAc) and crude methanol (MeOH). The nano- particles were done by followed the method of Dar and Soytong (2014), to yield Nano-CBH, Nano-CBE and Nano-CBM. Those nano-particles were evaluated to inhibit *Phytophthora* sp. The experimental design was used two factors factorial experiment in Completely Randomization. The experiment was repeated four times. Factor A was Nano-CBH, Nano-CBE and Nano- CBM. Factor B was 0, 1, 5 and 10 μg/ml. Each Nano-particle was dissolved in 2% dimethyl sulfoxide, and mixed to PDA before autoclaved at 121 ℃,15 lbs/inch^2 for 30 min. The culture of *Phytophthora* sp was cut at peripheral colony with sterilized cock borer (0.5 mm). Agar plug of *Phytophthora* sp was moved to the middle of PDA mixed with each nano-particles. All plates were incubated at room temperature until the pathogen in control growing full plate. The pathogen from each treatment were observed abnormal spores under compound microscope. Data were stistaically computed. The effective dose was calculated using probit analysis.

Results

Pathogenicity test

Pathogenicity test was conducted by detached leaves method which resulted durian leave var Montong showed brown hydrolysis expand around agar plug of pathogen. In control, leaves remained healthy as seen in Fig.1.

Dual culture test

Ch. brasiliense was proved it ability to inhibit plant pathogen *P. palmivora* causing disease of durian by using dual culture tests. The results showed that *Ch. brasiliense* gave significantly growth inhibition of *P. palmivora* which were 58.33% and showed significantly inhibited the spore production of pathogen of 88.82% (Table 1).

Figure1. Pathogenicity test of Phytophthora sp on Durian leaves

Testing crude extracts of Chaetomium brasilense to against Phytophthora spp in vitro

The crude extracts from *Ch. brasiliense* were used to test their abilities to control the growth of *P. palmivora*. The results showed that crude ethyl acetate (EtOAc) extract gave highest inhibition of *P. palmivora* colony growth which was 57.75% at the concentration of 1,000 µg/ml with the ED_{50} values of 204.28 µg/ml when compared to the control. Crude hexane and crude ethyl acetate (EtOAc) extract and showed significantly highest inhibition for the spore production of *P. palmivora* as 100% at the concentration of 1,000 µg/ml. Crude methanol extract gave the best to inhibit the trsed pathogen wghivh the ED50 was 5.94 ppm, and followed by crude ethyl acetate and crude hexane extracts which the ED50 values were 17.46 and 28.56 µg/ml., respectively (Table 2).

Table 1. Colony growth and number of spore on antagonistic dual-culture tests

Antaginistic fungi	***P. palmivora***			
	Colony diameter (cm)	Growth inhibition (%)	Spore number (10^4/ml)	Spore inhibition (%)
Control	9.00[a1]	-	27.43[a]	-
Ch. brasiliense	3.75[b]	58.33[b]	5.31[b]	88.82[a]
C.V. (%)	7.90		19.39	

[1] Means of four repeated experiments. Means followed by the same letters are not significantly differed by DMRT at P=0.01.

In vitro testing nano-particles from Chaetomium brasilense to control Phytohthora sp causing durian rot var Montong

Result from nano-particles showed high efficacy antifungal activity of nanoparticles from *Ch. brasilense* against *P. palmivora* The result of Nano particles from *Ch. brasilense* was showed that Nano-CBH, Nano-CBE and Nano-CBM gave highly significant inhibited the colony growth of *P. palmivora* as 90.00% which the ED_{50} values of 1.25, 1.12, 1.08 µg/ml., respectively and gave highly significant inhibition for the spore production of *P. palmivora* as 100% which the ED_{50} values of 8.68, 12.20 and 10.77ppm (Table 3).

Table 2. Crude extracts of *Ch. brasilense* testing for growth inhibition of *P. palmivora*

Crude extracts	Concentration (ppm)	Colony diameter (cm)[1]	Growth Inhibition(%)	ED_{50} (µg/ml)	Number of spores (10^7)	Inhibition (%)	ED_{50} (µg/ml)
Hexane	0	5.00[a]	-		1.475[a]	-	
	10	5.00[a]	0.00[g]		0.49[bc]	63.19[de]	
	50	5.00[a]	0.00[g]	770.60	0.53[bc]	63.87[de]	28.56
	100	5.00[a]	0.00[g]		0.39[cd]	72.13[cde]	
	500	3.29[c]	34.00[e]		0.17[efg]	87.90[abc]	
	1000	2.02[e]	57.75[c]		0.00[g]	100[a]	
Ethyl Acetate	0	5.00[a]	-		1.475[a]	-	
	10	5.00[a]	0.00[g]		0.63[b]	55.85[e]	
	50	5.00[a]	20.00[f]	204.28	0.32[cde]	78.17[bcd]	17.46
	100	3.37[e]	32.5[e]		0.31[cde]	78.29[bcd]	
	500	1.25[f]	75.00[b]		0.00[g]	100[a]	
	1000	0.86[g]	82.75[a]		0.00[g]	100[a]	
Methanol	0	5.00[a]	-		1.475[a]	-	
	10	5.00[a]	0.00[g]		0.48[bc]	65.88[de]	
	50	5.00[a]	0.00[g]		0.39[cd]	71.85[bcd]	
	100	5.00[a]	0.00[g]	-	0.23[efg]	83.51[bcd]	5.93
	500	4.12[b]	17.5[f]		0.13[efg]	91.40[ab]	
	1000	2.65[d]	47.00[d]		0.02[fg]	97.91[a]	
C.V. (%)		3.96			29.34		

[1] Means of four repeated experiments. Means followed by the same letters are not significantly differed by DMRT at P=0.01.

Table 3. Nano particle extracts of *Ch. brasilense* testing for growth inhibition of *P. palmivora*

Nano particles	Concentration (ppm)	Colony diameter(cm)[1]	Growth inhibition (%)	ED_{50} (µg/ml)	Number of spores (10^7)	Inhibition (%)	ED_{50} (µg/ml)
	0	5.00[a]	-		0.56[a]	-	
	1	2.75[b]	45.00[e]		0.22[b]	57.45[b]	
Nano-CBH	3	1.41[e]	71.75[cd]	1.25	0.00[c]	100[a]	8.68
	5	1.35[ef]	73.00[cd]		0.00[c]	100[a]	
	7	1.11[g]	77.75[bc]		0.00[c]	100[a]	
	10	0.5[h]	90.00[a]		0.00[c]	100[a]	
	0	5.00[a]	-		0.56[a]	-	
	1	2.60[bc]	47.50[e]		0.32[b]	36.67[c]	
Nano-CBE	3	1.67[d]	66.50[d]	1.12	0.00[c]	100[a]	12.20
	5	1.00[g]	80.00[b]		0.00[c]	100[a]	
	7	1.17[fg]	76.50[bc]		0.00[c]	100[a]	
	10	0.5[h]	90.00[a]		0.00[c]	100[a]	
	0	5.00[a]	-		0.56[a]	-	
	1	2.50[c]	50.00[e]		0.28[b]	47.95[bc]	
Nano-CBM	3	1.52[de]	69.50[d]	1.08	0.00[c]	100[a]	10.77
	5	1.12[fg]	77.50[bc]		0.00[c]	100[a]	
	7	0.5[h]	90.00[a]		0.00[c]	100[a]	
	10	0.5[h]	90.00[a]		0.00[c]	100[a]	
C.V. (%)		8.74			51.16		

[1] Means of four repeated experiments. Means followed by the same letters are not significantly differed by DMRT at P=0.01.

Discussion

Morphological study was done in previous experiment to prove identification of *P. palmivora* which cultured on potato dextrose agar. The colony is white, slow growing, non septate mycelia, sporangia produce readily and abundantly. The identity in morphological characteristics are consistent with descriptions of *Phytophthora palmivora* (Erwin and Ribiero, 1996).

The results showed that crude ethyl acetate (EtOAc) extract from *Ch. brasilense* gave highest inhibition of *P. palmivora* colony growth which was 57.75% at the concentration of 1,000 µg/ml with the ED_{50} values of 204.28 ppm when compared to the control. As similar in the previous report, found that crude extract of *Ch. globosum* CG05, *Ch. cupreum* CC3003, *Ch. lucknowense* CL01 showed ability to inhibit mycelial growth and spore production of *P. palmivora* PHY02 in laboratoty test (Hung *et al.*, 2015b). This result is also similar reported by Hung *et al.*, (2015a) found that crude extracts of these *Chaetomium* species exhibited antifungal activities against mycelial growth of *P. nicotianae*, with effective doses of 2.6~101.4 µg/ml.

It was found that nano-particles derived from *Ch. brasilense* was actively inhibited the *P. palmivora*. Nano-CBH, Nano-CBE and Nano-CBM gave highly significant inhibition of the colony growth of *P. palmivora* at 90.00% and gave highly significant inhibition for the spore production of *P. palmivora* at 100%. This result is similar reported by Thongkham (*et al.*, 2017) found that nano-particles derived from *Ch. cupreum* to inhibit mycelial growth and spore production of *Phytophthora* spp causing root rot in durian. This study was also similar to Dar *et al*. (2013) that nano particles of *Ch. globosum* and *Ch. cuperum* were proved to againt *F. oxysporum* f.sp. *lycopersici* and *Colletotrichum capsici*.

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